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Influence of salinity and various nitrogen sources on cell growth and lipid production of the green microalga *Micractinium pusillum* Fresen.

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ABSTRACT

Nitrogen is considered one of the most important nutrients for the growth of microalgae which affects cell development and metabolic processes, leading to the increase of lipid content of the growing cell under the deficient concentration of nitrogen. At a given concentration of 9.89 mM, the impact of six different nitrogen sources, including urea, glycine, NH_4Cl, $NaNO_3$, KNO_3 and yeast extract, were compared to study how nitrogen sources affect cell growth and lipid content of the green microalga *Micractinium pusillum*. Yeast extract was found to be the best nitrogen source for both biomass and lipid productivity (0.1 g L^{-1} d^{-1} and 5.5 µgml^{-1} day^{-1}، respectively). *M. pusillum* prefers nitrate in the form of KNO_3 and $NaNO_3$ rather than other organic nitrogen such as urea and glycine. Laboratory results indicated that ammonium supplementation resulted in the inhibition of the growth of *M. pusillum*, in addition, 30 mM Sodium chloride was the optimal concentration for obtaining quantitatively high total lipid content. In conclusion, this work highlighted that saturated fatty acids increased by 95% and 75% when *M. pusillum* was grown at 30 mM NaCl and in the yeast extract culture, respectively.

INTRODUCTION

Microalgal biomass production is influenced by many physicochemical parameters, including nutrients, temperature, light, pH, and salinity **(Kim *et al.*, 2014; Yen *et al.*, 2014; Bartley *et al.*, 2016; El-Sheekh *et al.*, 2022)**. Nitrogen is regarded as one of the most important nutrients for growth since nitrogen is a component for both structural and functional proteins, including peptides, energy transfer molecules, genetic materials, chlorophylls and enzymes of algal cells, and genetic materials in algal cells **(Cai *et al.*, 2013; Hu, 2013).** The concentration of nitrogen in the culture medium significantly impacts the rate of cell growth and the biochemical contents of microalgae**. (Wang *et al.*, 2013).** The stress response caused by nitrogen deficiency is a natural mechanism by which microalgae can alter lipid metabolism. Despite the fact that nitrogen deficiency appears to inhibit the cell cycle and the production of nearly all cellular components, the rate of lipid synthesis remains higher, resulting in the accumulation of oil in starved cells

(El-Sheekh and Abomohra, 2012). Several studies have found that when the nitrogen is limited in the culture medium, microalgae slow down the cell growth rate and increase their lipid or carbohydrate content, lowering protein synthesis **(Ho *et al*., 2014)**.

Most microalgae can consume a broad range of nitrogen sources, such as urea and glycine, as well as nitrate, nitrite, and ammonium **(Becker, 1994; El-Sheekh *et al*., 2020)**. Each nitrogen supply is first transformed into ammonium and then digested into amino acids through various processes (**Cai *et al*., 2013)**. Most microalgae have been known to favor ammonium as converting it into amino acids uses less energy, however, some species prefer nitrate for growth, including *Botryococcus braunii* and *Dunaliella tertiolecta*. **(Chen *et al*., 2011; Ruangsomboon, 2015).** For growth, some *Chlorella* species prefer nitrate to ammonium, and they successfully employ sources of organic nitrogen that contain peptone, yeast extract (YE), glycine, and urea **(Li *et al*., 2013; Muthuraj *et al*., 2014)**. The supply of nitrogen also affects the lipid content; for example, supplementing with ammonium increases *Chlorella sorokiniana* lipid content by over two folds compared to urea or nitrate **(Wan *et al*., 2012)**.

Salinity also has a significant impact on lipids accumulation in the microalgal cell. Microalgae vary in their ability to adapt to salt stress depending on the degree of their tolerance. They are divided into two groups: halophilic (requiring salt for optimal growth) and halotolerant (having reaction mechanisms that allow for their existence under salt stress), and in any situation, the microalgae develop certain metabolites to protect themselves from salt damage and to maintain osmotic balance **(Richmond, 1986)**.

This work aims to study different nitrogen sources on the green microalga *Micractinium pusillum* growth and improvement of lipid productivity. Also، the work focus on how *M. pusillum* adapts to various concentrations of NaCl and how those variables affect growth and lipid and fatty acids accumulation.

MATERIALS AND METHODS

Microalgal strain growth condition

The green microalgae *Micractinium pusillum* was provided by the National institute of Oceanography and Fisheries, Alexandria, Egypt. In 1L Erlenmeyer flasks stopped with cotton plugs, 800 ml of Khul medium (**Khul and Lorenzen, 1964**) was used. The flasks were sterilized in an autoclave at 121 ^{o}C and 1.5 atoms for 20 minutes. After cooling, a specific volume of *Micractinium pusillum* pre-cultures was added to the Erlenmeyer flasks. Aeration was delivered to the culture through silicon tubes with one end connected to the culture flask and the other end connected to the aerator. Algal culture flasks were cultured with atemperature of 25 $^{\circ}$C ±2 and continuous fluorescent light at 45 mole $m^{-2}s^{-1}$.

Different nitrogen sources include Urea ($CO(NH_2)_2$, 0.6 g L-1), Sodium nitrate ($NaNo_3$, 0.85 g L^{-1}), Glycine (NH_2CH_2COOH, 0.75 g L^{-1}), Yeast extract (YE) (Initial nitrogen concentration was the same, at 9.89 mM 138.5 mg N L^{-1}) and ammonium chloride (NH_4Cl, 0.53 g L^{-1}), were used in medium instead of potassium nitrate (KNO_3 1 g L^{-1}) in the control. To study the effect of salt stress, *M. pusillum* was exposed to sodium chloride with different concentrations (10, 20, 30, and 40 mM).

Measurement of cell growth

Optical density

A spectrophotometer was used to measure the optical density at 680 nm every two days to determine the algal culture's growth. The obtained absorbance was then used to plot the algal culture's growth curve.

Dry weight estimation

A particular volume of the algal suspension from the exponential growth phase was centrifuged at 4000 rpm for 15 minutes; it was gently washed with distilled water. The precipitated biomass was dried in pre-weighed Petri plates overnight at 100 ^oC in an oven until constant weight. Data were supplied by $g.L^{-1}$ **(Ahmed and Osman, 1973).**

Biomass productivity estimation

The previous explanation of **Abomohra *et al*. (2013)** was used to estimate the biomass productivity according to Eq. (1).

$$Biomass\ productivity\ BP;\ (g\ L^{-1}\ day^{-1}) = CDW_L - CDW_E / (t_L - t_E) \quad \text{Eq. (1)}$$

Where CDW_E (g L^{-1}) is the cellular dry weight at the early exponential phase (t_E), and CDW_L is the cellular dry weight at the late exponential phase (t_L).

Total lipids and productivity estimation

The Park method was modified to directly measure lipid content using the Sulfophosph vanallin (SPV) reaction. The phosphovanillin reagent was first made by stirring continuously until 0.06 g of vanillin was dissolved in 10 ml of deionized water. Then, the combination received 40 ml of concentrated phosphoric acid. Using a known quantity of biomass, the biomass was centrifuged at 4000 RPM for 5 minutes to obtain the biomass. After being treated with 2 mL of concentrated sulfuric acid (98%) for 10 minutes at 100 °C in a water bath, the sample was dried. After cooling to room temperature, the reaction mixture was added to 5 ml of freshly produced phospho-vanillin reagent. The combination was then incubated for 15 min at 37 °C in the incubator. After that, a pink colour appeared, and the sample's lipid content was calculated by measuring the pink color's absorbance at 530 nm with a spectrophotometer **(Jaeyeon Park *et al*., 2016)**. The standard curve was used to determine the amount of lipid curve, and the lipid concentration was given as (μg ml^{-1}). Lipid productivity was determined using Eq. (2) as previously mentioned by **Abomohra *et al*. (2013)**.

$$Lipid\ productivity\ (LP;\ (\mu g\ L^{-1}\ day^{-1}) = LC_L - LC_E / (t_L - t_E) \quad \text{Eq. (2)}$$

Where LC_E is the lipid content (μg ml^{-1}) at the early exponential phase (t_E) and LC_L (μg ml^{-1}) is the lipid content at the late exponential phase (t_L).

Extraction of total lipids

Extraction of the total lipid content was obtained from the modified Folch approach **(Folch *et al.*, 1957)** after making several adjustments. 50 ml of an algal culture were centrifuged at 4500 rpm for 15 minutes before 40 ml of a chloroform/methanol (2/1, v/v) solution was added to the cells. After being shaken at 120 rpm for 48 hours at room temperature, the mixture was filtered through filter paper (Whatman No. 1) to separate the liquid phase. A separatory funnel was used to separate the liquid phase into upper and lower layers, after the liquid phase had been moved to a new flask and washed with a 0.9 percent NaCl (w/v) solution. The bottom phase containing the lipids was taken in a pre-weighed glass vial and dried at 40 °C for two days in order to evaporate the solvent. The amount of total lipid content is shown by the difference between W2 and W1.

Fatty acids profiles estimation

According to the modified procedure of **Zahran and Tawfeuk (2019)**, 15 mg of oil was mixed with 1.0 mL of n-hexane before being mixed with 1.0 mL of sodium methoxide (0.4 mol) to create fatty acid methyl esters. The mixtures were vortexed for 30 seconds and then allowed to settle for 15 minutes. Gas chromatography was used to recover and analyze the upper phase that contained the FAMEs (GC-FID). The GC analysis was performed using a Perkin Elmer Auto System XL with a flame ionization detector (FID). The capillary column was a fused silica DB-Wax (60m x 0.32mm i.d.) The oven's temperature was set to start at 150 °C and climb to 220 °C at a rate of 3 °C/min. The carrier gas was helium at a flow rate of 1.1 ml/min. The injector and detector had temperatures of 230 and 250 °C, respectively.

Statistical analysis

The data were expressed as the mean and standard deviation (SD) of three replicates. The gathered data were statistically assessed using the SPSS 23.0 program using one-way analysis of variance (ANOVA), then Duncan's multiple range testing for data with a significant difference, at p 0.05.

RESULTS

Influence of different nitrogen sources on the microalga's growth, dry weight, and biomass productivity

Depending on the optical density (OD 680 nm), the yeast extract recorded the highest growth (2.11), followed by KNO_3 as control 1.89, $NaNo_3$1.55, urea 0.72, glycine 0.64, and ammonium chloride 0.07) as shown in **Fig. 1**.

Among the different nitrogen sources used, YE also recorded the highest dry weight and biomass productivity (1.22 g L^{-1} and 0.1 g L^{-1} d^{-1}, respectively), followed by control (0.93 g L^{-1} and 0.07 g L^{-1} d^{-1}, respectively), $NaNO_3$ (0.76 g L^{-1} and 0.06 g L^{-1} d^{-1},

respectively), urea (0.64 g L^{-1} and 0.06 g L^{-1} d^{-1}, respectively), glycine (0.59 g L^{-1} and 0.05 g L^{-1} d^{-1}) and ammonium chloride (0.19 g L^{-1} and 0.015 g L^{-1} d^{-1}, respectively) respectively as shown in **Figs. 2 and 3.**

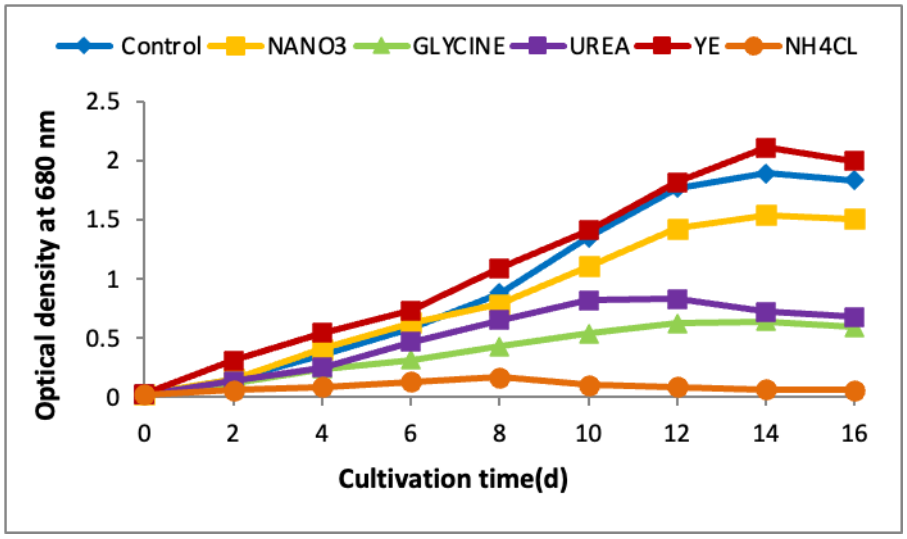

Fig.1. Growth of *Micractinium pusillum* grown under different nitrogen sources determined by measuring optical density at 680 nm.

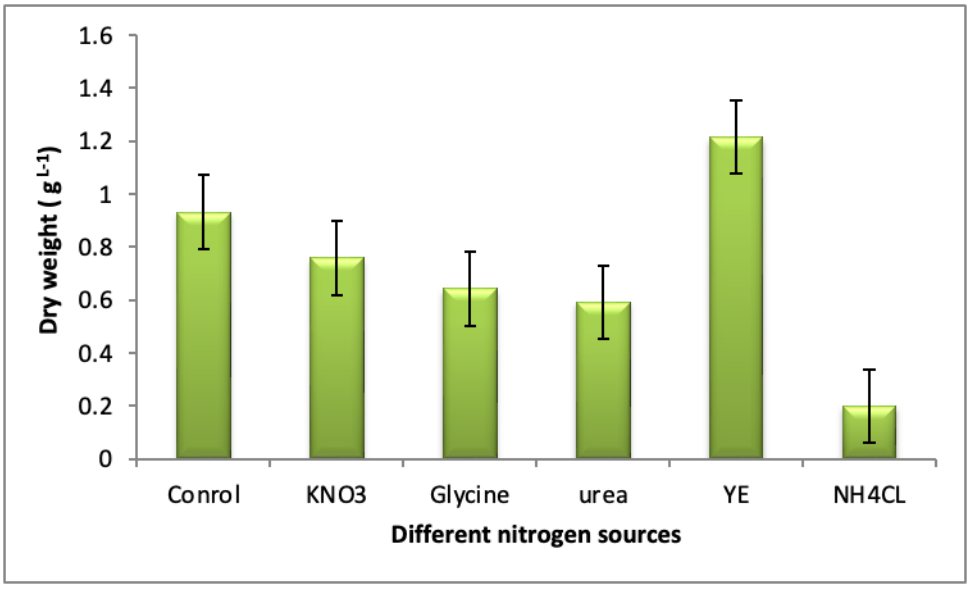

Fig.2. Cellular dry weight of *Micractinium pusillum* under different nitrogen sources

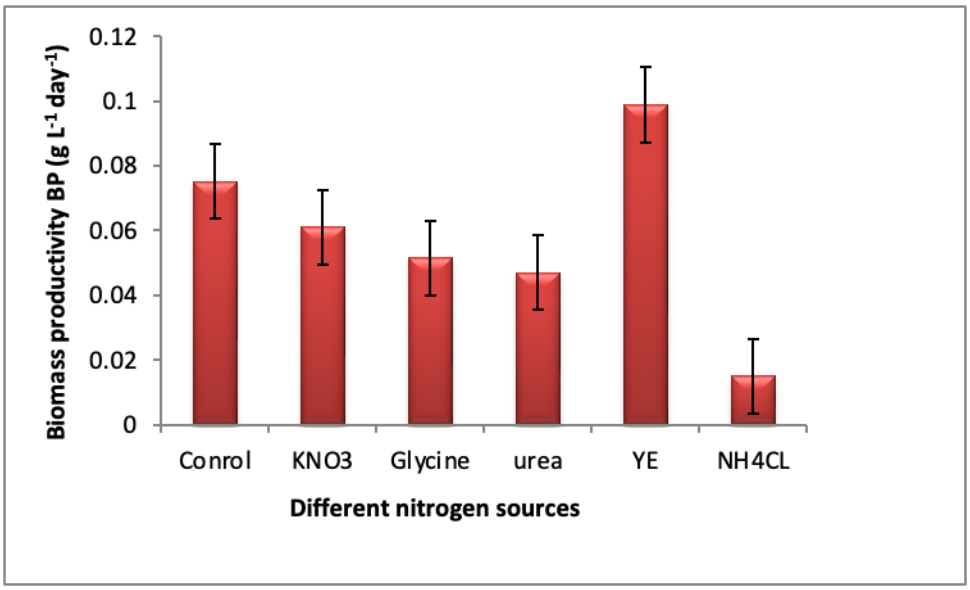

Fig.3. Biomass productivity of *Micractinium pusillum* under different nitrogen sources

Total lipid content and lipid productivity under different nitrogen sources

The obtained results in **Table 1** reveal that the highest total lipid content and lipid productivity were observed with the YE as a nitrogen source followed by control. Both glycine and $NaNO_3$ have the same lipid productivity, but glycine has a lipid content higher than $NaNO_3$. Urea comes in fifth, and NH_4CL showed the lowest total lipid content and lipid productivity.

Table 1. Effect of different nitrogen sources on total lipid content and lipid productivity of *Micractinium pusillum*

Nitrogen sources	Total lipid (μgml^{-1})	lipid productivity (μgml^{-1} day^{-1})
Control	$28.21^{B}\pm0.41$	$2.25^{B}\pm0.05$
$NANO_3$	$14.91^{C}\pm0.08$	$1.19^{C}\pm0.008$
Glycine	$15.92^{C}\pm0.17$	$1.19^{C}\pm0.01$
Urea	$9.55^{D}\pm0.22$	$0.74^{D}\pm0.02$
YE	$67.83^{A}\pm1.01$	$5.50^{A}\pm0.08$
NH_4CL	$6.16^{E}\pm1.01$	$0.47^{E}\pm0.08$
F value	8317.44^{***}	4231.14^{***}

Data are expressed as the mean ± standard deviation (SD) of three replicates. Different letters represent the statistical comparisons between groups using one-way ANOVA and post hoc Duncan's test ($p<0.05$).

Effect of salt stress on microalgae growth, dry weight, and biomass productivity. Different concentrations of sodium chloride (10, 20, 30, and 40 mM) were used as stress conditions on the growth of *M. pusillum*. The concentration of 30 mM NaCl recorded the highest growth measures as OD at 680 (2.29), followed by 20, control, 10, and 40 mM NaCl (1.96, 1.89, 1.05, and 0.98, respectively), as shown in **Fig.4**.

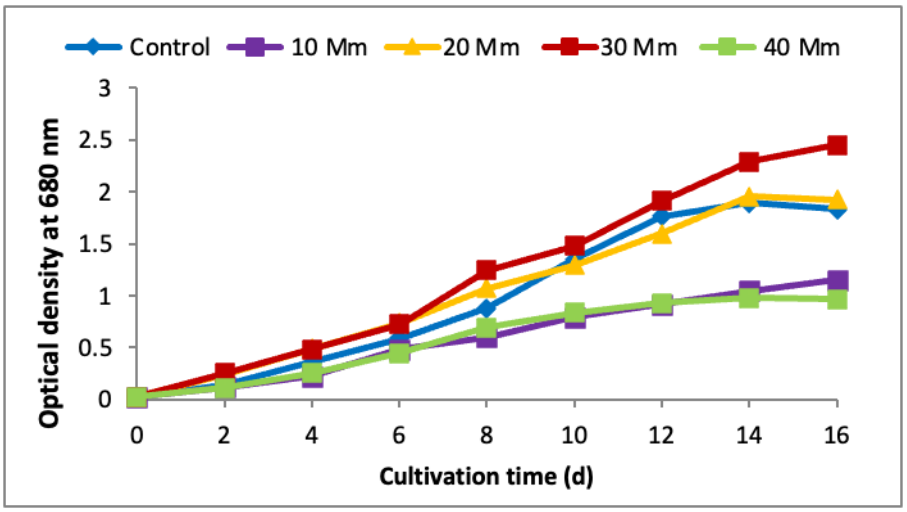

Fig.4. Growth of *Micractinium pusillum* under different concentrations of NaCl determined by measuring optical density at 680 nm.

The highest dry weight and biomass productivity were observed at 30 mM (1.25 g L^{-1} and 0.1 g L^{-1} d^{-1}, respectively), followed by 20 mM (1.19 g L^{-1} and 0.09 g L^{-1} d^{-1}, respectively), control (0.93 g L^{-1} and 0.07 g L^{-1} d^{-1}, respectively), 10 mM (0.90 g L^{-1} and 0.07 g L^{-1} d^{-1}, respectively) and 40 mM (0.56 g L^{-1} and 0.04 g L^{-1} d^{-1}, respectively) **(Figs. 5 and 6)**.

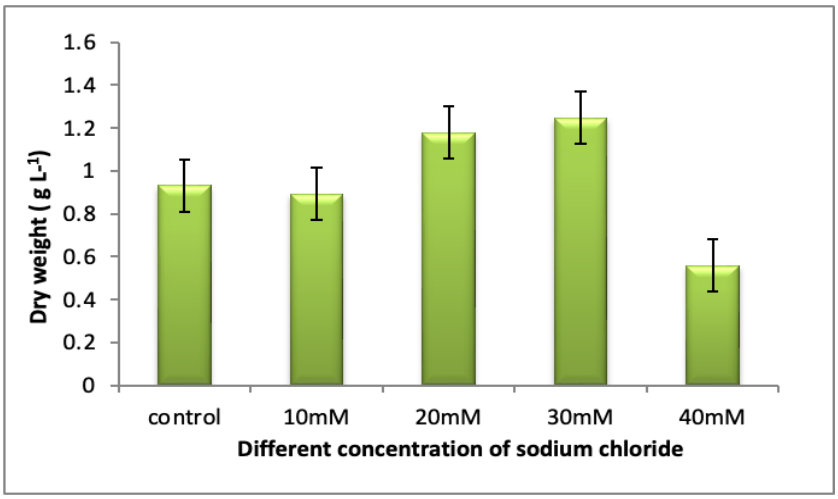

Fig.5. Cellular dry weight of *Micractinium pusillum* under different concentrations of NaCl

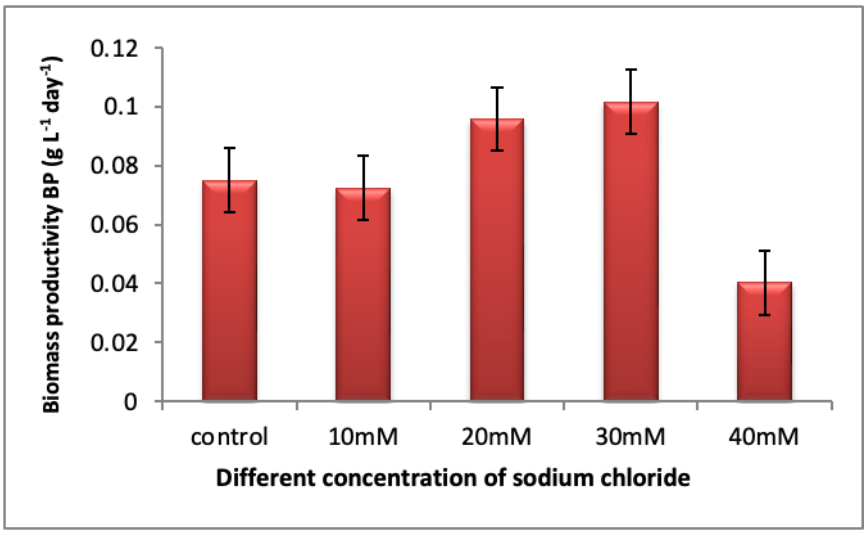

Fig.6. Biomass productivity of *Micractinium pusillum* under different concentrations of NaCl

Influence of salt stress on the total lipid content

Data is recorded in **Table 2**. Show the highest total lipid content and lipid productivity at concentrations of 20 and 30 mM compared to control.

Table 2. Effect of different sodium chloride on total lipid content and lipid production of *Micractinium pusillum*

Sodium chloride concentration	Total lipid content (μgml^{-1})	lipid productivity (μgml^{-1} day^{-1})
Control	$28.21^{C}\pm0.41$	$2.25^{C}\pm0.05$
10 mM	$25.1^{D}\pm0.29$	$2^{D}\pm0.02$
20 mM	$31.78^{B}\pm0.27$	$2.58^{B}\pm0.03$
30 mM	$36.28^{A}\pm0.1$	$2.91^{A}\pm0.02$
40 mM	$23.01^{E}\pm0.15$	$1.93^{E}\pm0.02$
F value	1114.39^{***}	635.78^{***}

Data are expressed as the mean± standard deviation (SD) of three replicates. Different letters represent the statistical comparisons between groups by using one-way ANOVA and post hoc Duncan's test ($p<0.05$).

Fatty acids content

Table 3. Fatty acids profiles of *M. pusillum* cultivated under control culture and yeast extract and sodium chloride (30 mM)

Fatty acid	C-number	Control	Yeast extract	30 mM
SFAs				
Tridecylic acid	C13:0	0.269	.....	.....
Palmitic acid	C16:0	17.75	17.28	28.69
Margaric acid	C17:0	0.289	2.389	.....
Stearic acid	C18:0	0.808	0.7	4.15
Arachidic acid	C20:0	1.77	16.44	7.68
Tricosanoic acid	C23:0	0.358	0.443	1.66
MUSFAs				
palmitelaidic acid	C16:1t	3.89	.....	.....
palmitoleic acid	C16:1n7	.....	2.609	.....
cis-10-Heptadecenoic acid	C17:1	.....	6.01	14.39
Oleic acid	C18:1(n-9)	18.45	12.16	.....
cis-Methyl 11-eicosenoate	C20:1	4.5	.....	.....
PUFAs				
7,10-Hexadecadienoic acid, methy	C16:2	1.81	.....	2.16
7,10,13-Hexadecatrienoic acid, m	C17:3	5.92	.....	5.45
Methyl 4,7,10,13-hexadecatetrae	C17:4	6.85	4.85	.....
Linoleic acid	C18:2(n-6)	11.6	11.53	11.48
Gamma-linlenic acid (GLA)	C18:3(n-6)	0.335	0.148	.....
Alpha-linolenic acid(ALA)	C18:3n3	24.03	22.98	24.35
Stearidonic acid	C18:4n3	.....	0.97	.....
Eicosatrienoic acid	C20:3n3	.....	0.5	.....
Arachidonic acid	C20:4n6	1.36		
Eicosapentaenoic acid(EPA)	C20:5n3	.....	0.627	.....
cis-13,16-Docasadienoic acid	C22:2	.....	0.354	.....
Total SFAs		**21.24**	**37.26**	**42.17**
Total MUFAs		**26.84**	**20.78**	**14.39**
Total PUFAs		**51.91301402**	**41.96**	**43.44**

SFA = saturated fatty acids, USFA = unsaturated fatty acid, MUSFA = Mono-unsaturated fatty acid, PUSFA = Poly-unsaturated fatty acid.

The results illustrated in **Table 3** show that saturated fatty acids (SFAs) increased by 98% at 30 mM sodium chloride with a percentage (42.17%) and 75% when the alga was grown on nitrogen source (YE) with a percentage (37.26%). Palmitic acid (C16:0), Stearic acid (C18:0), and arachidic acid (C20:0) were the major saturated fatty acids found in both control and YE cultures. Oleic acid (C18:1) (ω9) was the most dominant

monounsaturated fatty acids (MUFAs), with the highest percentage of 18.45% shown in the control culture. Polyunsaturated fatty acids are higher in the control culture with a percentage (51.91%), and omega3 (ω3) fatty acids are found as Alpha-linolenic acid (ALA) (C18:3), Stearidonic acid (C18:4), Eicosatrienoic acid (C20:3) and Eicosapentaenoic acid (EPA) (C20:5) in YE culture, while it found as only Alpha-linolenic acid (ALA) in control culture and 30 mM NaCl. The culture of control was recorded with the highest percentage of ω6 (13.92%), while the percentage of ω6 was 11.67% and 11.48% in YE culture and 30 mM NaCl, respectively.

DISCUSSION

Micractinium pusillum grown in yeast extract as a nitrogen source showed the highest biomass and lipid productivity, this could be attributed to the presence of a wide range of substances, like vitamins, peptides, carbs, and amino acids, present in YE. In the study of **Garam *et al.* (2016)**, it was noted that nitrogen is not solely responsible for the highest cell growth rate and lipids content that was reached. Through mixotrophic metabolism, *M. pusillum* may use both the nitrogen and other organic substances present in YE. Freshwater microalga *Scenedesmus acutus* actively utilizes YE and exhibits extremely high cell concentration of up to 10.4 g L^{-1}, but *Chlorella* sp. exhibits a little rise in cell concentration **(Gu *et al.*, 2015; EL-Mohsnawy *et al.*, 2020)**. According to another study, YE dramatically raised *Tetraselmis* sp. cell concentrations within two days, and after 10 days, it peaked at 2.23 g L^{-1} **(Garam *et al.*, 2016)**.

Even though *M. pusillum* was supplemented with urea and continued to develop successfully, the final cell concentration and lipid yield were lower than they were when nitrate was present. On the other hand, it was discovered that the marine microalga *Nannochloropsis salina* clearly chose urea over nitrate or ammonium for growth **(Campos *et al.*، 2014)**. Additionally, *Isochrysis galbana* reportedly produces the highest growth in urea as opposed to nitrate or nitrite (**Fidalgo *et al.* 1998)**.

M. pusillum showed the lowest biomass when glycine was employed, this was because *M. pusillum* lacked the necessary enzymatic N-assimilation machinery for the metabolism of amino-acid once it had entered the cell or the metabolic adaption process required more time **(Berland *et al.*, 1979)**, but glycine comes third in total lipids production, and this may be due to at 14^{th} of cultivation, lipid accumulated because microalgal's cells adapted to the presence of glycine and assimilated it.

The observation that the addition of ammonium chloride inhibited cell development indicates that the 9.89 mM concentration was hazardous for cell growth. The excessive ammonium transfer to the cells may prevent the chloroplast from producing ATP, which would then prevent photosynthesis, the process that has an inhibitory influence on cell growth **(Ramanna *et al.*, 2014**). Furthermore, in some circumstances, too much

ammonium in the medium can significantly release H^+ ions and drop pH resulting in inhibition of cell growth and may even result in cell lysis **(Li *et al*., 2013; Wu *et al*., 2013).**

Biomass and lipids content increased at concentrations of 20 and 30 mM, and this might be a result of the organism's adaptation to these concentrations. This adaptation may result from the existence of salt-tolerant enzymes that improve ATPase function **(López-Pérez *et al*., 2009)** and/or enhance the growth and metabolic processes of some microalgae **(Talukdar *et al*., 2012)**. **Ranga Rao *et al*. (2007)** also reported that at salinity (17 mM to 85 mM), biomass, carbohydrate, and carotenoids of *Botryococcus braunii* were higher than in control. According to **Hart *et al*. (1991)**, lower growth rates at higher salt concentrations are caused by a decline in photosynthetic rate.

Saturated fatty acids (SFAs) in YE culture were higher than control culture, while polyunsaturated fatty acids (PUFAs) were reduced by 19%. Palmitic acid (C16:0), Stearic acid (C18:0), and arachidic acid (C20:0) were the major saturated fatty acids found in both control and YE cultures. It was reported that saturated fatty acids (SFAs) of *Tetraselmis* sp. increased by 23% when it grew mixotrophically under YE, while polyunsaturated fatty acids (PUFAs) were higher with a percentage (44.33%) when *Tetraselmis* sp. grown in autotrophic culture with sodium nitrate **(Garam *et al*., 2016; El-Sheekh and El-Kassas 2016)**.

At 30 mM NaCl, saturated fatty acids (SFAs) are dramatically increased by 98%; these results are, to some extent, in agreement with the data obtained by **Atikij *et al*. (2019)**, who demonstrated that Chlorophyta under stress tended to synthesize saturated fatty acids (SFA). Significant variations can occur in the kinds of fatty acids partitioned into TAG following receipt of salt stress; this can be referred to the diverse phylogeny of microalgae. In the present study, polyunsaturated fatty acids (PUFAs) were reduced by 19%. In contrast, **Atikij *et al*. (2019**) found that ochrophyta (diatoms) chose to produce polyunsaturated fatty acids (PUFA) when exposed to salt stress.

One study found that monounsaturated fatty acids (MUFA), whereas ochrophyta (diatoms) chose to produce polyunsaturated fatty acids (PUFA) when exposed to salt stress.

CONCLUSION

In conclusion, the results show that species differ in their preferred nitrogen source and their ability to utilize nitrogen, and some species, including *M. pusillum*, which would benefit better from mixotrophic cultivation employing YE, as demonstrated in the current study. Also, *M. pusillum* can adapt to NaCl concentrations (10 mM to 40 mM), and the optimal concentration was 30 mM which enhanced both growth and lipids.

REFERENCES

Abomohra, A.; Wagner, M., El-Sheekh, M. and Hanelt, D. (2013). Lipid and total fatty acid productivity in photoautotrophic freshwater microalgae: screening studies towards biodiesel production. J. Appl. Phycol. 25: 931–936.

Ahmed, A and Osman, M.E. (1973). The influence of light on 14C02 -fixation by synchronous cultures of *Chlorella pyrenoidosa*. Egypt. J. Bot. 16: 319-327.

Atikij, T.; Syapurti, Iwahashi H.; Praneenararat T.; Sirisattha S.; Kageyama H.; Waditee-Sirisattha R. (2019). Enhanced lipid production and molecular dynamics under salinity stress in green microalga *Chlamydomonas reinhardtii* (137C). Mar. Drugs 17: 484.

Bartley, M. L.; Boeing, W. J.; Daniel, D.; Dungan, B. N. and Schaub, T. (2016). Optimization of environmental parameters for *Nannochloropsis salina* growth and lipid content using the response surface method and invading organisms. J. Appl. Phycol. 28:15-24.

Becker, E. W. (1994). Microalgae biotechnology and microbiology. Cambridge University Press, New York, 18 pp

Berland, B.R.; Bonin, D.J.; Gurin-Ancey, O. and Antia, N.J. (1979). Concentration Requirement of Glycine as Nitrogen Source for Supporting Effective Growth of Certain Marine Microplanktonic Algae Marine Biology 55: 83-92.

Cai, T.; Park, S. Y. and Li, Y. (2013). Nutrient recovery from wastewater streams by microalgae: status and prospects. Renew. Sustain. Energy Rev. 19:360-369.

Campos, H.; Boeing, W. J.; Dungan, B. N and Schaub, T. (2014). Cultivating the marine microalga *Nannochloropsis salina* under various nitrogen sources: effect on biovolume yields, lipid content and composition, and invasive organisms. Biomass Bioenergy 66:301-307.

Chen, M.; Tang, H.; Ma, H.; Holland, T. C.; Ng, K. Y. and Salley, S. O. (2011). Effect of nutrients on growth and lipid accumulation in the green algae *Dunaliella tertiolecta*. Bioresour. Technol. 102:1649-1655.

EL-Mohsnawy E., El-Sheekh MM., Mabrouk M., Zoheir W. (2020). Enhancing accumulation of omega 3 and 9 fatty acids in *Chlorella vulgaris* under mixotrophic nutrition. The Journal of Animal and Plant Sciences 30(2): 485-492.

El-Sheekh, M.M and Abomohra, A.E.F. (2012). Optimization of biomass and fatty acid productivity of *Scenedesmus obliquus* as a promising microalga for biodiesel production. World J Microbiol Biotechnol. DOI 10.1007/s11274-012-1248-2.

El-Sheekh, M.M., El-Kasas, H. (2016). Induction of the synthesis of bioactive compounds of the marine alga *Tetraselmis tetrathele* (West) Butcher grown under salinity stress. Egyptian Journal of Aquatic Research. 42(4): 385-391.

El-Sheekh M., Hassan, L.H., Morsi H.H. (2020). Growth enhancement of *Spirulina platensis* through optimization of media and nitrogen sources. Egyptian Journal of Botany. 61(1): 61-69.

El-Sheekh M.M., Saman JM., Grmasha RA., Abdul-Adel E., Saleh, MM., Al-sareji O.J. (2022). Influence of Fe^{+2} on the biomass, pigments, and essential fatty acids of *Arthrospira platensis*. Biomass Conversion and Biorefinery https://doi.org/10.1007/s13399-022-02470-9.

Fidalgo, J.P., Cid, A.; Torres, E.; Sukenik, A and Herrero, C. (1998). Effects of nitrogen source and growth phase on proximate biochemical composition, lipid classes and fatty acid profile of the marine microalga *Isochrysis galbana*. Aquaculture 166:105-116.

Folch, J.; Lees, M and Stanley, G. (1957). A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem. 226:497–509.

Garam, K.; Ghulam, M and Kisay, L. (2016). Effects of nitrogen sources on cell growth and biochemical composition of marine chlorophyte *Tetraselmis* sp. for lipid production Algae 2016, 31(3): 257-266

Gu, H.; Nagle, N., Pienkos, P. T. and Posewitz, M. C. (2015). Nitrogen recycling from fuel-extracted algal biomass: residuals as the sole nitrogen source for culturing *Scenedesmus acutus*. Bioresour. Technol. 184:153-160.

Hart, B.T.; Bailey, P.; Edwards, R.; Hortlek, K.; James, K.; McMohan, A.; Meredith, C and Swading, K. (1991). A review of the salt sensitivity of the Australian fresh water biota. Hydrobiologia 210: 105–144.

Ho, S. -H.; Ye, X.; Hasunuma, T.; Chang, J. -S. and Kondo, A. (2014). Perspectives on engineering strategies for improving biofuel production from microalgae: a critical review. Biotechnol. Adv. 32:1448-1459.

Hu, Q. (2013). Environmental effects on cell composition. In Richmond, A. & Hu, Q. (Eds.) Handbook of Microalgal Culture: Applied Phycology and Biotechnology. 2nd ed. Wiley Blackwell, West Sussex, pp. 114-122.

Jaeyeon, P.; Hae. J.; Eun, Y. Yand Seung, J. M. (2016). Easy and rapid quantification of lipid contents of marine dinoflagellates using the sulpho-phospho-vanillin method. Algae 2016, 31(4): 391-340.

Kim, G.; Mujtaba, G.; Rizwan, M. and Lee, K. (2014). Environmental stress strategies for stimulating lipid production from microalgae for biodiesel. Appl. Chem. Eng. 25:553-558.

Kuhl, A. and Lorenzen, H. (1964). Handling and culturing of *Chlorella*. In: Prescott, D.M. (ed.), Methods in cell physiology. Acad Press NY and London, 1: 152–187.

Li, T.; Zheng, Y.; Yu, L and Chen S. (2013). High productivity cultivation of a heat-resistant microalga *Chlorella sorokiniana* for biofuel production. Bioresour. Technol. 131:60- 67.

Li, T.; Zheng, Y.; Yu, L. and Chen, S. (2013). High productivity cultivation of a heat-resistant microalga *Chlorella sorokiniana* for biofuel production. Bioresour. Technol. 131:60- 67.

López-Pérez, L.; Martínez-Ballesta, M.; Maurel, C and Carvajal M. (2009). Changes in plasma membrane lipids, aquaporins and proton pump of broccoli roots, as an adaptation mechanism to salinity. Phytochem. 70:492–500.

Muthuraj, M.; Kumar, V.; Palabhanvi, B. and Das, D. (2014). Evaluation of indigenous microalgal isolate *Chlorella sp*. FC2 IITG as a cell factory for biodiesel production and scale up in outdoor conditions. J. Ind. Microbiol. Biotechnol. 41:499-511.

Ramanna, L.; Guldhe, A.; Rawat, I and Bux, F. (2014). The optimization of biomass and lipid yields of *Chlorella sorokiniana* when using wastewater supplemented with different nitrogen sources. Bioresour. Technol. 168:127-135.

Ranga Rao, A., Dayananda, C., Sarada, R., Shamala T.R and Ravishanka.G.A. (2007). Effect of salinity on growth of green alga *Botryococcus braunii* and its constituents Bioresource Technology, 98: 560–564.

Richmond, A. (1986). Cell response to environmental factors. In: Richmond, A. (Ed.), CRC Handbook of Microalgal Mass culture. CRC Press Inc., Florida, pp. 89–95.

Ruangsomboon, S. (2015). Effects of different media and nitrogen sources and levels on growth and lipid of green microalga *Botryococcus braunii* KMITL and its biodiesel properties based on fatty acid composition. Bioresour. Technol. 191:377-384.

Talukdar, J.; Kalita, M.C and Goswami, B.C. (2012). Effects of salinity on growth and total lipid content of the biofuel potential microalga *Ankistrodesmus falcatus* (Corda. Ralfs. Int J Sci Eng Res. 3:1–7.

Wan, M. -X.; Wang, R. -M.; Xia, J. -L.; Rosenberg, J. N.; Nie, Z. -Y.; Kobayashi, N.; Oyler, G. A. and Betenbaugh, M. J. (2012). Physiological evaluation of a new *Chlorella sorokiniana* isolate for its biomass production and lipid accumula- tion in photoautotrophic and heterotrophic cultures. Biotechnol. Bioeng. 109:1958-1964

Wang, J.; Sommerfeld, M. R.; Lu, C. and Hu, Q. (2013). Combined effect of initial biomass density and nitrogen concentration on growth and astaxanthin production of *Haematococcus pluvialis* (Chlorophyta) in outdoor cultivation. Algae 28:193-202.

Wu, L.F.; Chen, P.C and Lee, C.M. (2013). The effects of nitrogen sources and temperatures on cell growth and lipid accumulation of microalgae. Int. Biodeterior. Biodegrad. 85:506-510.

Yen, H. W.; Hu, I. C.; Chen, C. Y and Chang, J. S. (2014). Design of photobioreactors for algal cultivation. In Pandey, A., Lee, D. -J., Chisti, Y. & Soccol, C. R. (Eds.) Biofuels from Algae. Elsevier, San Diego, CA, pp. 23-45.

Zahran, H.A and Tawfeuk, H.Z (2019). Physicochemical properties of new peanut (*Arachis hypogaea* L.) varieties. *OCL* 26: 19.